Case No. 410.015

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	MIDOUX, P. et al.		
Serial No.	:	09/297,519	Group Unit :	1633
Filed	:	May 3, 1999	Examiner :	Nguyen, D.
For	:	NOVEL POLYMERIC COMPLEXES FOR THE TRANSFECTION OF NUCLEIC ACIDS, WITH RESIDUES CAUSING THE DESTABLIZATION OF CELL MEMBRANES		

Statement Under 37 C.F.R. §1.821(f) or §1.825(b)

Commissioner of Patents
Washington, D.C. 20231

Dear Sir:

I hereby certify that:

[x] The paper Sequence Listing submitted herewith and computer readable Sequence Listing attached hereto are identical (37 C.F.R. §1.821(f)).

[ ] The substitute paper Sequence Listing and substitute computer readable Sequence Listing submitted herewith are identical. No new matter is included (37 C.F.R. §1.825(b)).

Respectfully submitted,

BIERMAN, MUSERLIAN AND LUCAS, L.L.P.

Date: 4-26-01

By: _____
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend the paragraph beginning on page 3, line 30 and ending on page 4, line 10 with the following:

--However, the use of even defective viral particles presents safety problems. Adenoviruses induce a very strong immune response after injection with the complexes.

c) Peptides which are permeabilizing and/or fusiogenic in a slightly acid medium are used as auxiliaries to promote passage of DNA into the cytosol. These are mainly peptides of 20 amino acids derived from virus fusion proteins, such as, for example, the *N*-terminal peptide of the sub-unit HA2 of the haemagglutinin of the influenza virus, or synthetic peptides, such as GALA (SEQ ID NO:1), an oligomer containing several recurring units of Glu-Ala-Leu-Ala (SEQ ID NO:1). These peptides are most often used in the free form (that is to say not covalently bonded) with the DNA/polylysine complexes. The efficiency of peptides is greatly reduced in the presence of serum in the cell culture medium, which restricts their use to experiments *in vitro* or to *ex vivo*. Some peptides covalently bonded to DNA/polylysine complexes are still effective in promoting transmembrane passage of DNA, while others lose their permeabilizing power after bonding.--

Please amend the paragraph section beginning on page 19, line 20 to page 20, line 13, with the following:

--B) Peptides

a. anti-inflammatory peptides or certain of their fragments recognized by receptors of the vascular wall, such as

-vasodilator intestinal polypeptide (VIP)

HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2
(SEQ ID NO:2)

-atrial natriuretic polypeptide (ANP)
SLRRSSCFGGRMDRIGAQSGLGCNSFRY
(SEQ ID NO:3)

-lipocortin
HDMNKVLDL (SEQ ID NO:4)

-bradykinin
RPPGFSPFR (SEQ ID NO:5);

b. ligand peptides of integrins, such as peptides containing the sequence RGD, fibronectin ligand;

c. chemiotactic factors, such as formyl-peptides and their antagonists: FMLP, (N-formyl-Met-Leu-Phe);

d. peptide hormones, such as α-MSH: Ac-SYSMEHFRWGKPV-NH_2 (SEQ ID NO:6) and their antagonists.--

Please amend the paragraph beginning on page 41, line 28 to page 42, line 10, with the following:

--The DNA/HispLK complexes are formed by mixing the plasmid pCMVLUC (10 μg in 0.7 ml DMEM) and the polylysine substituted by 70 histidyl residues (40 μg in 0.3 ml DMEM). After 30 minutes at 20°C, the solution containing the complexes is diluted once with DMEM and topped up with 5% f[o]etal bovine serum. The DNA/pLK complexes are formed by mixing the plasmid pCMVLUC (10μg in 0.7 ml DMEM) and the polylysine (5 μg in 0.3 ml in DMEM). After 30 minutes at 20°C, the solution containing the complexes is diluted once with DMEM and topped up with 5% f[o]etal bovine serum and either with 100 μM chloroquine (+ chloro) or 20 μM of a fusiogenic peptide (+

E5CA) (GLFEAIAEFIEGGWEGLIEGCA; SEQ ID NO:7). The medium in which the HepG2 cells (3 x 10^5 cells/4 cm^2) have grown for 24 hours is removed and replaced by a solution (1 ml) containing a DNA/polymer complex (5 μg/ml DNA). After incubation for 4 hours at 37°C, the cell medium is removed again and the cells are incubated in culture medium in the presence of 10% f[o]etal bovine serum. The expression of the gene of luciferase was determined 48 hours after the transfection by measuring the luminescence emitted (RLU: relative values of the light emitted expressed in arbitrary units) in the cell lysates for 4 seconds.--

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EXHIBIT 4